

## EnzyChrom™ Glutamate Assay Kit (EGLT-100)

### Quantitative Colorimetric Determination of Glutamate at 565 nm

#### DESCRIPTION

Glutamate is an important chemical in general metabolism. It is also a crucial mammalian neurotransmitter that is believed to be involved in a number of neurological and psychiatric disorders such as lateral sclerosis, lathyrism, autism and Alzheimer's disease. Glutamate is also widely used as a flavor enhancer in the food industry.

Simple, direct and automation-ready procedures for measuring glutamate concentration are very desirable. BioAssay Systems' EnzyChrom™ glutamate assay kit is based on glutamate dehydrogenase catalyzed oxidation of glutamate, in which the formed NADH is coupled to the formazan (MTT)/phenazine methosulfate (PMS) Reagent. The intensity of the product color, measured at 565 nm, is proportionate to the glutamate concentration in the sample.

#### APPLICATIONS

**Direct Assays:** glutamate in serum, plasma, tissue extracts and food extract samples.

**Drug Discovery/Pharmacology:** effects of drugs on glutamate levels.

#### KEY FEATURES

**Sensitive and accurate.** Detection limit of 50  $\mu\text{M}$ , linearity up to 2.5 mM glutamate in 96-well plate assay.

**Convenient.** The procedure involves adding a single working reagent, and reading the optical density at time zero and at 30 min at room temperature. No 37°C heater is needed.

**High-throughput.** Can be readily automated as a high-throughput 96-well plate assay for thousands of samples per day.

#### KIT CONTENTS (100 tests in 96-well plates)

<b>Assay Buffer:</b> 10 mL	<b>NAD Solution:</b> 1 mL
<b>PMS Solution:</b> 1.5 mL	<b>MTT Solution:</b> 1.5 mL
<b>Enzyme:</b> 120 $\mu\text{L}$	<b>Standard:</b> 1 mL 100 mM Glutamate

**Storage conditions.** Store all reagents at -20°C. Shelf life of at least 6 months (see expiry dates on labels).

**Precautions:** reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

#### PROCEDURES

- Calibration Curve.** Prepare 600  $\mu\text{L}$  2.5 mM Glutamate Premix by mixing 15  $\mu\text{L}$  100 mM Standard and 585  $\mu\text{L}$  distilled water. Dilute standard as follows. Transfer 20  $\mu\text{L}$  standards into wells of a clear bottom 96-well plate.

No	Premix + H <sub>2</sub> O	Vol ( $\mu\text{L}$ )	Glutamate (mM)
1	100 $\mu\text{L}$ + 0 $\mu\text{L}$	100	2.5
2	80 $\mu\text{L}$ + 20 $\mu\text{L}$	100	2.0
3	60 $\mu\text{L}$ + 40 $\mu\text{L}$	100	1.5
4	40 $\mu\text{L}$ + 60 $\mu\text{L}$	100	1.0
5	30 $\mu\text{L}$ + 70 $\mu\text{L}$	100	0.75
6	20 $\mu\text{L}$ + 80 $\mu\text{L}$	100	0.5
7	10 $\mu\text{L}$ + 90 $\mu\text{L}$	100	0.25
8	0 $\mu\text{L}$ + 100 $\mu\text{L}$	100	0

**Samples:** add 20  $\mu\text{L}$  sample per well in separate wells. **IMPORTANT:** Serum and tissue extract samples require a sample blank.

- Reagent Preparation.** Spin the Enzyme tube briefly before pipetting. For each well of reaction, prepare Working Reagent by mixing 50  $\mu\text{L}$  Assay Buffer, 1  $\mu\text{L}$  Enzyme, 10  $\mu\text{L}$  NAD, 14  $\mu\text{L}$  PMS and 14  $\mu\text{L}$  MTT. Fresh reconstitution is recommended. *Where a sample blank is required, prepare a Blank Working Reagent by mixing 50  $\mu\text{L}$  Assay Buffer, 10  $\mu\text{L}$  NAD, 14  $\mu\text{L}$  PMS and 14  $\mu\text{L}$  MTT (i.e. No Enzyme).*

- Reaction.** Add 80  $\mu\text{L}$  Working Reagent (or Blank Working Reagent where appropriate) per reaction well quickly. Tap plate to mix briefly and thoroughly.
- Read optical density (OD<sub>0</sub>) for time "zero" at 565 nm (520-600nm) and OD<sub>30</sub> after a 30-min incubation at room temperature.
- Calculation.** Subtract OD<sub>0</sub> from OD<sub>30</sub> for the standard and sample wells. Next, subtract the  $\Delta\text{OD}_{\text{water}}$  (Std 8) from each  $\Delta\text{OD}_{\text{standard}}$  and  $\Delta\text{OD}_{\text{sample}}$  to obtain the  $\Delta\Delta\text{ODs}$ . (*Where a sample blank was required, subtract the  $\Delta\text{OD}_{\text{blank}}$  from  $\Delta\text{OD}_{\text{sample}}$  to obtain the  $\Delta\Delta\text{OD}_{\text{sample}}$ .)* Plot the  $\Delta\Delta\text{OD}_{\text{standard}}$ 's and use this standard curve to convert the  $\Delta\Delta\text{OD}_{\text{sample}}$  values to sample glutamate concentration.

$$[\text{Glutamate}] = \frac{\Delta\Delta\text{OD}_{\text{SAMPLE}}}{\text{Slope}} \quad (\text{mM})$$

Note: If the sample  $\Delta\Delta\text{OD}$  values are higher than the  $\Delta\Delta\text{OD}$  value for the 2.5 mM glutamate standard, dilute sample in distilled water and repeat this assay. Multiply the results by the dilution factor.

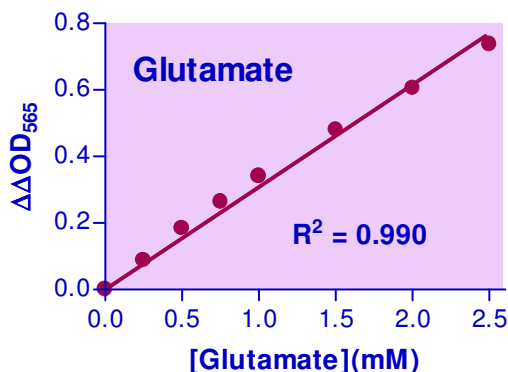
**Conversions:** 1 mM glutamate = 14.5 mg/dL.

#### MATERIALS REQUIRED, BUT NOT PROVIDED

Pipeting (multi-channel) devices. Clear-bottom 96-well plates (e.g. Corning Costar) and plate reader.

#### GENERAL CONSIDERATIONS

- This assay is based on an enzyme-catalyzed kinetic reaction. Addition of Working Reagent should be quick and mixing should be brief but thorough. Use of multi-channel pipettor is recommended.
- The following substances interfere and should be avoided in sample preparation: ascorbic acid, SDS (>0.2%), sodium azide, NP-40 (>1%) and Tween-20 (>1%).



Standard Curve in 96-well plate assay

#### LITERATURE

- Perez-de la Mora, M, et al (1989). A Glutamate Dehydrogenase-Based Method for the Assay of L-Glutamic Acid: Formation of Pyridine Nucleotide Fluorescent Derivatives. *Anal. Biochem.* 180: 248-252.
- Matsumura, H. and Miyachi S (1980). Cycling assay for nicotinamide adenine dinucleotides. *Methods Enzymol.* 69: 465-470.
- Graham, LT and Aprison, MH (1966). Fluorometric determination of aspartate, glutamate, and gamma-aminobutyrate in nerve tissue using enzymic methods. *Anal. Biochem.* 15: 487-497.